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TITLE: Understanding the Mechanisms of Platelet Alloimmunization and Its Prevention

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14. ABSTRACT We have used a dog platelet transfusion model to evaluate methods of preventing alloimmune platelet refractoriness. Our initial hypothesis was that filtration-leukoreduction, when combined with y-irradiation, would prevent alloimmunization. However, this approach was successful in only 2/11 recipient dogs (18%). Over the past year, we have switched our approach to determine if a pathogen reduction process involving adding riboflavin to the platelets followed by UV-irradiation (mirasol technology), when combined with filtration leukoreduction, would be successful. We have been able to prevent alloimmune platelet refractoriness using this approach in 13/14 (93%) of recipient dogs.					
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INTRODUCTION:

Over the last year, we have evaluated a pathogen-reduction process which is able to inactivate antigen-presenting white cells to determine whether this technology, when used alone or combined with filtration leukoreduction, would be able to prevent alloimmune platelet refractoriness in our dog platelet transfusion model. We have collaborated in these studies with the CaridianBCT Corporation which adds riboflavin (Vitamin B₂) to the platelets and then exposes the platelets to UV-light (Mirasol Technology). This process prevents replication of DNA and RNA in viruses, bacteria, and WBCs and has been shown to inactivate a broad range of pathogens as well as prevent transfusion-associated graft-versus-host disease eliminating the need for γ -irradiation of transfused blood products.

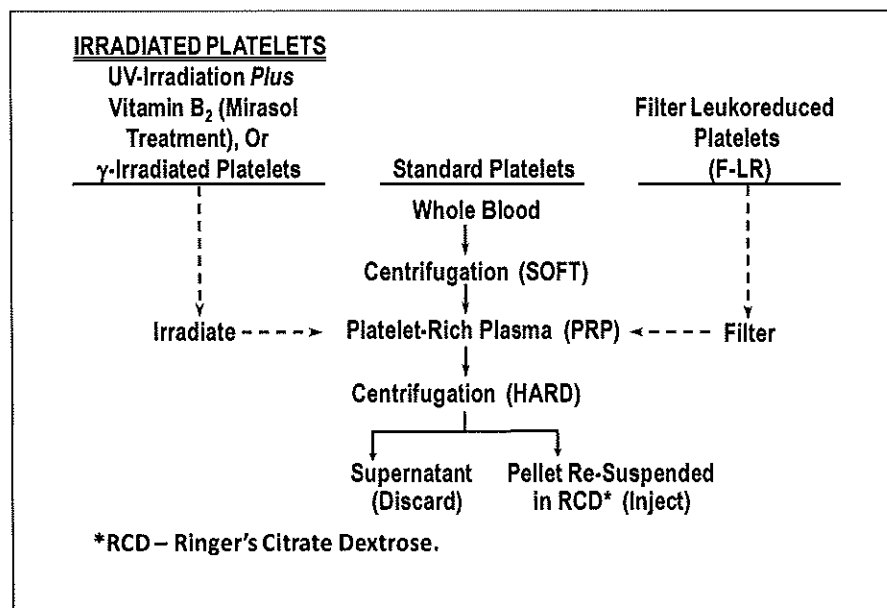
BODY:

Methods.

Experimental Design of the Platelet Transfusion Experiments.

- 1) Select crossmatch negative and DLA-DRB mismatched donor/recipient pairs.
- 2) Platelets are obtained weekly from a single random donor.
- 3) Donor dog's platelets are unmodified (standard), filter-leukoreduced, γ -irradiated, addition of Vitamin B₂ to the platelets *plus* UV-irradiation (Mirasol technology), or treatments are combined.
- 4) Donor dog's platelets are radiochromium labeled prior to recipient transfusion.
- 5) Serial blood samples are drawn from the recipient to determine recovery and survival of the donor dog's platelets.
- 6) Baseline and weekly blood samples are drawn to identify IgM and IgG antibodies to donor platelets and WBCs. Samples are also tested against autologous platelets and WBCs as a negative control. Antisera from alloimmune platelet refractory animals are pooled and run as a positive control against both autologous and allogeneic platelets and WBCs.
- 7) Recipient receives up to 8 weekly transfusions from their donor or until they become platelet refractory.
- 8) **Primary Endpoint:** Platelet refractoriness is defined as $\leq 5\%$ of the radiolabeled donor dog's platelets still circulating in the recipient at 24 hours post-transfusion after two sequential transfusions.
- 9) Autologous radiolabeled platelet recovery and survival measurements are performed baseline and at the end of each study. These measurements are done to ensure that refractoriness to donor transfusions is caused by alloimmunization rather than by some condition in the transfused recipient that would affect the recovery and survival of both their allogeneic as well as their autologous platelets.

Preparation of Donor Platelets.



Results.

Table 1

	# Donors Accepted / # Recipients (%)
Standard (Unmodified Platelets)	1 / 7 (14%)
<u>Filtration:</u>	
● Pall PL1B Filter	1 / 5 (20%)
● Fenwal PLS-5A Filter	4 / 6 (66%)
Mirasol Treatment	1 / 8 (13%)
γ -Irradiation	0 / 5 (0%)

Table 2

FILTER LEUKOREDUCTION PLUS γ -IRRADIATION OR MIRASOL TREATMENT

	<u>γ-IRRADIATION</u>	<u>MIRASOL TREATMENT</u>
<u>Filtration</u>	<u># Donors Accepted / # Recipients (%)</u>	<u># Donors Accepted / # Recipients (%)</u>
Pall PL1B*	0 / 5 (0%)	6 / 6 (100%)
Fenwal PLS-5A**	2 / 6 (33%)	7 / 8 (88%)
Total	2 / 11 (18%)	13 / 14 (93%)

* PL1B Only – 1 / 5 (20%).

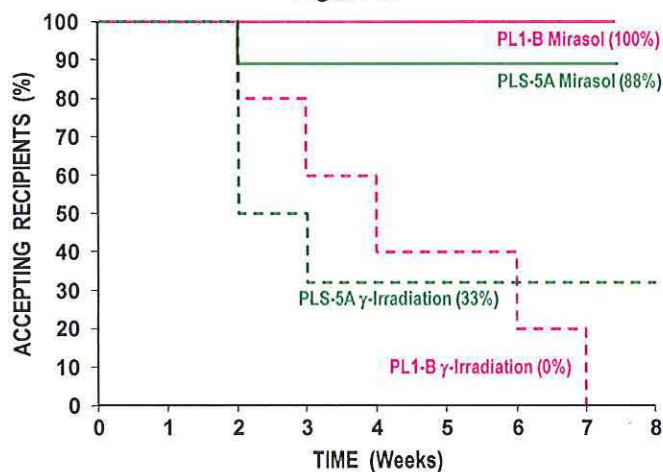
** PLS-5A Only – 4 / 6 (66%).

Figure 1

Figure 1 is a Kaplan-Meier survival plot showing the percentage of recipients accepting the graft over 8 weeks for five treatment groups. The y-axis is labeled 'ACCEPTING RECIPIENTS (%)' and ranges from 0 to 100. The x-axis is labeled 'TIME (Weeks)' and ranges from 0 to 8. The groups and their final acceptance percentages are: Filtration PLS-5A (66%, red solid line), Mirasol Rx (13%, blue solid line), Standard (14%, orange dashed line), Filtration PL1-B (20%, green solid line), and γ -Irradiation (0%, pink dashed line). The plot shows that the Filtration PLS-5A group has the highest acceptance rate, while the γ -Irradiation group has the lowest (0%).

Group	Final Acceptance (%)
Filtration PLS-5A	66%
Mirasol Rx	13%
Standard	14%
Filtration PL1-B	20%
γ -Irradiation	0%

Figure 2



KEY RESEARCH ACCOMPLISHMENTS:

-5-

understanding of how to prevent this adverse event. We know that F-LR does not remove all antigen-presenting WBCs. F-LR may remove enough WBCs to allow the remaining WBCs to be effectively inactivated using the Mirasol technology allowing the combined approach to be successful. The fact that this combined approach is effective even with a poorly performing filter may suggest that this combined approach may be successful when used with any available filter.

It cannot be emphasized enough the many advantages of this combined approach for transfused recipients in addition to preventing alloimmunization. Leukoreduction prevents CMV transmission by transfusion, reduces cytokine reactions from WBC breakdown during storage, and prevents febrile transfusion reactions. Pathogen reduction inactivates bacteria, viruses, and other transfusion-transmissible agents, may prevent the introduction of the next unknown pathogen into the blood supply, and can eliminate the need for γ -irradiation of blood products to prevent transfusion associated graft-*versus*-host disease.

REPORTABLE OUTCOMES:

Please refer to the attached abstract which has been accepted as a poster presentation at the annual American Society of Hematology meeting in December 2010.

CONCLUSION:

- Different filters have different alloimmunization rates, possibly related to the WBCs they remove *versus* those that remain following filtration.
- Neither γ -irradiation nor pathogen-reduction (Mirasol technology) are able to prevent platelet alloimmunization.
- γ -irradiation does not increase acceptance of F-LR platelets.
- Mirasol treatment *plus* F-LR is almost completely effective in preventing alloimmune platelet refractoriness.
- If Mirasol-treated/F-LR platelets were given to immunosuppressed patients, it is anticipated that alloimmunization would be completely prevented.

REFERENCES:

None.

APPENDICES:

Appendix 1: 2010 American Society of Hematology Abstract:

Slichter SJ, Pellham E, Bailey SL, Christoffel T, Gaur L, Latchman Y, Nelson K, Bolgiano D. Prevention of alloimmune platelet refractoriness in a dog model requires both removal and inactivation of contaminating donor white blood cells. Abstract accepted for poster presentation at the 2010 ASH annual meeting.

APPENDIX 1

2010 American Society of Hematology Abstract #28030

Prevention of Alloimmune Platelet (Plt) Refractoriness In a Dog Model Requires Both Removal and Inactivation of Contaminating Donor White Blood Cells (WBCs)

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Background: The TRAP Trial (NEJM 1997;337:1861) evaluated filtration leukocyte-reduction (F-LR) and UV-B irradiation (UV-BI) to prevent plt alloimmunization in AML patients (pts) receiving chemotherapy. Inclusion of UV-BI was based on a 45% rate of preventing plt alloimmunization in our dog plt tx model. UV-BI was 79% successful in the TRAP Trial. The lower success rate in the dog was probably because we use normal immunocompetent recipient dogs *versus* chemotherapy-induced immunosuppressed pts. The residual rate of alloimmunization in the TRAP Trial using either F-LR or UV-BI was still 17% to 21%, suggesting that better prevention methods are needed. Methods: In our dog plt tx model, we evaluated two leukoreduction filters to remove antigen presenting WBCs (APCs) and γ -irradiation (γ -I) or UV-I combined with riboflavin (Mirasol pathogen reduction technology) to inactivate APCs. Crossmatch-negative, DLA-DRB incompatible donor/recipient pairs were used. Tx's from the same donor were given weekly for up to 8 weeks or until the donor's ⁵¹Cr-labeled plts were rejected based on two sequential tx's with donor plt recoveries $\leq 5\%$ at 20 hours post-tx.

Results:

Platelet Modification	# Donors Accepted / # Recipients (%)
None	1 / 7 (14%)
<u>Single Modification:</u>	
<u>F-LR:</u>	
Pall PL1-B Filter	1 / 5 (20%)
Fenwal PLS-5A Filter	4 / 6 (66%)
γ -I	0 / 5 (0%)
Mirasol Treatment	1 / 8 (13%)
<u>Combined Modifications:</u>	
<u>F-LR plus γ-I:</u>	
PL1-B Filter	0 / 5 (0%)
PLS-5A Filter	2 / 6 (33%)
Total*	2 / 11 (18%)
<u>F-LR plus Mirasol Treatment:</u>	
PL1-B Filter	6 / 6 (100%)
PLS-5A Filter	7 / 8 (88%)
Total*	13 / 14 (93%)

*F-LR plus Mirasol-treated plts had a total success rate of 93% *versus* F-LR, γ -I plts at 18% (p=0.005).

Residual WBC counts for standard, γ -I, or Mirasol-treated plts averaged $2,860/\mu\text{l} \pm 1,800/\mu\text{l}$, and, for F-LR plts using the PL1-B or the PLS-5A filter, they were $80/\mu\text{l} \pm 10/\mu\text{l}$ and $110/\mu\text{l} \pm 270/\mu\text{l}$, respectively. Residual WBCs were significantly reduced after F-LR compared to non-leukoreduced plts (p<0.001), but there was no significant difference for WBC removal using either filter (p=0.86).

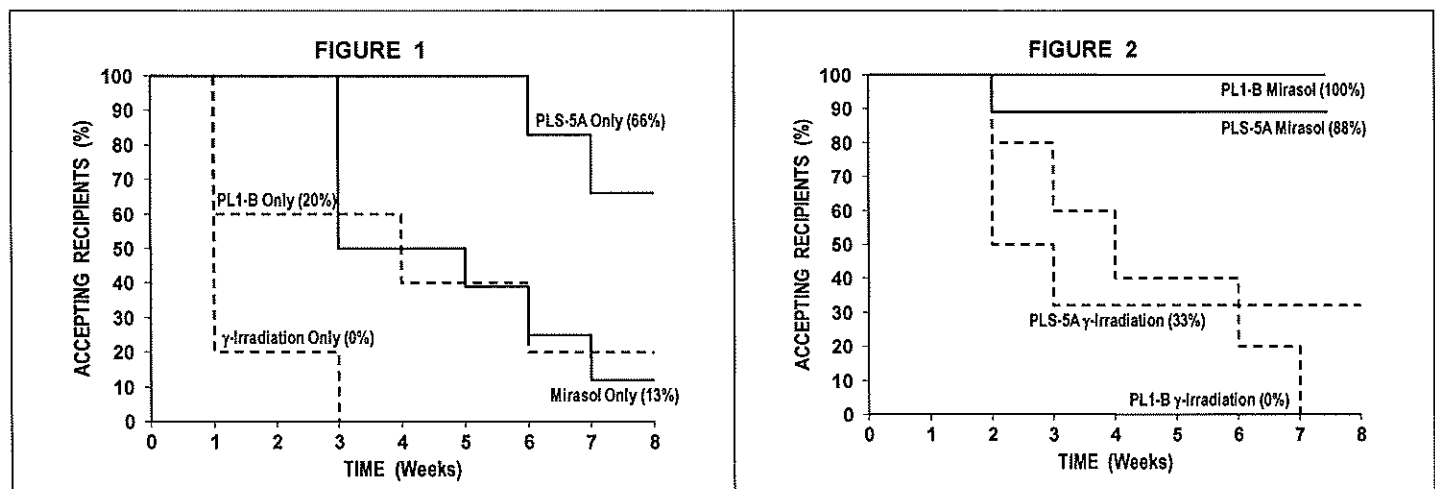


Figure 1 shows the time to alloimmune plt refractoriness for recipients of single modified plts either F-LR, γ -I, or Mirasol-treated, while Figure 2 shows similar data for dogs who received F-LR plts combined with either γ -I or Mirasol treatment. Conclusions: Except for the results with the PLS-5A filter, a single modification of the donor dog's plts is only minimally successful. Although residual WBC counts were similar for both filters, the better results achieved with the PLS-5A filter compared to the PL1-B filter (66% *versus* 20% success rate, respectively) suggest that the types of WBCs removed may be as important as simply a quantitative reduction in WBCs. Unfortunately, adding γ -I to F-LR did not improve the results using either filter. In contrast, Mirasol treatment markedly improved the results with both filters, suggesting that the effects of F-LR and Mirasol treatment are synergistic with F-LR removing APCs, while Mirasol treatment inactivates residual APCs. The effect is particularly striking for the PL1-B filter that is only minimally effective when used alone. The fact that a combined F-LR Mirasol treatment approach is so successful in our immunocompetent dog model using both good and poor performing filters may suggest two things: 1) the combined approach will be equally successful in both immunosuppressed as well as non-immunosuppressed pts; and 2) using any available leukoreduction filter combined with Mirasol treatment will give results similar to the two filters already tested. Based on our prior experience with UV-BI, data from our dog model may be directly transferable to man.